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Thrombin protease-activated receptor inhibitors from the peel of *Ananas comosus* (L.) Merr.: an *in silico* approach

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Abstract

Background: Mortality and morbidity related to coronary atherothrombotic diseases and the unpredictable adverse effect of available anticoagulant drugs prompt the need for the development of effective and safe therapeutic agents. This study assessed the metabolomic profiling and molecular docking studies of the constituents of the unripe peel fruit of *Ananas comosus* (L.) Merr. methanolic extract against thrombin protease-activated receptors (PARs). **Methods:** Metabolomics profiling of the methanolic extract of the unripe peel of *A. comosus* was carried out using gas chromatography connected with a mass spectrometer (GC/MS). Molecular docking was done to assess the affinity of the identified compounds for the active sites of PARs, and the binding behaviors were visualized with DS BIOVIA. pkCSM, a web server, screened two probable compounds which presented ideal binding with all the receptors. **Results:** The GC/MS profiling showed a total of 12 volatile compounds with benzyl alcohol being the most prominent compound. The molecular docking analysis showed 2-(4-methylphenyl)-indolizine, and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose demonstrated optimal binding with the selected PARs. The computed pharmacokinetic and pharmacodynamics properties of the selected compounds presented 2-(4-methylphenyl)-indolizine possesses drug-like properties. **Conclusion:** The findings of this study could be explored and optimized in the development of safe and efficient plant-based anti-thrombotic agents.

Keywords: *A. comosus*, Thrombin, Molecular docking, Protease-Activated Receptors

1. Introduction

Blood is a connective tissue in humans and other vertebrates flowing through the blood vessels smoothly and efficiently to deliver to cells needed materials including oxygen and nutrients. However, this smooth flow is obstructed by thrombus resulting in coronary atherothrombotic diseases that lead to death (WHO, 2017). Other clot formation diseases include pulmonary embolism, cerebrovascular accident (CVA), myocardial, and cerebral infarction (Ashorobi and Fernandez, 2019). Atherothrombotic coronary artery disease and deep vein thrombosis are major underlying death drives worldwide (Herrington *et al*., 2016). To substantiate this, Gryka *et al*. (2017) reported that 17 million deaths are caused yearly by cardiovascular events, and 7.3 million are caused by ischemic heart disease while 6.2 million of deaths are caused by strokes. This report was validated in WHO (2017) report whereby 31% of global death was due to these collective cardiovascular disorders and most occurs in low and middle-income countries.

Thrombosis is a fatal disease involving the blood clots formation which leads to associated coronary diseases in the circulatory system due to homeostasis imbalance (Ko et al., 2004; Mahmud et al., 2015). Mumaw et al. (2015) reported the crucial cellular component of arterial thrombin as platelet aggregation with evidence from different studies showing Protease-Activated Receptors (PARs) as thrombin activities' mediator that enhances human platelet activation. PARs, examples of G-protein-coupled receptors (GPCR) family, expressed in different cell types cause proteolytic cleavage at the N-terminal sequence for activation (Coughlin, 2000; Hollenberg and Compton, 2002). The cleavage remainers bind intra-molecularly to induce intracellular signal transduction that promotes thrombosis via receptor activation (Adams et al., 2011). Human PAR1, PAR3, and PAR4 have been known for their significant role in blood coagulation via interaction with thrombin (Ma et al., 2005), a known platelet agonist generated by coagulation system (Covic et al., 2002). Moreover, Charlotte et al. (2019) reported that platelet aggregation with platelet adhesion and activation is known to be a vital pathogenic factor in the development of atherosclerosis and associated thrombosis in humans via receptor-ligand interaction. Anticoagulants have been used in the prevention and management of cardiovascular disorders that are associated with thrombosis due to their clot formation inhibiting potential (De Caterina et al., 2013; da Silva and Ferreira, 2015). Several anticoagulant drugs such as heparin and warfarin are available to suppress atherothrombotic events; however, these compounds might not be healthy alternatives, besides being expensive and producing a wide spectrum of adverse effects (Gryka et al., 2017; Wong et al., 2017). This has prompted the search for novel cost-effective antithrombotic agents that are less toxic (Lau et al., 2009). Plants from time immemorial have been known to be the

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promising sources of novel drug candidates for the prevention and treatment of diseases including blood-clotting disorders. Some plant materials such as Alium cepa, Panax notoginseng, and Orbignya phalerata had been studied for their repositioning feasibility as anticoagulant agents for management and handling of thrombotic disorders (Azevedo et al., 2007; Shikha et al., 2014). Numerous investigations have been done on the putative effect of some phytoconstituents against platelet aggregation, towards increase fibrinolysis and coronary atherothrombotic diseases (CADs) as a whole (Yoo et al., 2014; Lee et al., 2015; Mohd Nor et al., 2016; Oso et al., 2019). Moreover, the peels and seeds of plant materials such as Lycopersicum esculentum Mill., Curcuma longa L., and Ananas comosus (L.) Merr. are examples of plant materials that had been reported to be prospective sources of pharmacologic agents against thrombosis (Evangelista et al., 2012).

Ananas comosus is a fruit that belongs to the family of Bromeliaceae. The inedible parts of the fruit such as the peels, crown, and core have been reported to be rich sources of beneficial biologically active phytochemicals such as polyphenols (Li *et al*., 2014). Also, Li *et al*. (2014) identified catechin, epicatechin, ferulic acid and gallic acid as the phytochemical constituents of the peel of *A. comosus* methanolic extract through HPLC-MS analysis. The classes of these phytocompounds are known to contribute immeasurably to various pharmacological properties of plant materials (Banji *et al*., 2018; Abdel-Mawgoud *et al*., 2019). Therefore, this study aimed at investigating the putative anti-thrombotic effects of the phytocompounds of the unripe peel fruit of *A. comosus* methanolic extract through *in silico* studies.

2. Material and Methods

2.1. Plant Materials

The plant materials (unripe pineapple fruit) were obtained from local suppliers in Ajebo, Ogun State, South-Western Nigeria and authenticated at the Department of Biological Sciences, McPherson University, Nigeria.

2.2. Methodology

2.2.1. Extraction of plant materials

The peel of fruit was removed, washed three times with distilled water, and dried at room temperature of 30±1˚C. The dried peel was pulverized and reserved for subsequent extraction. Fifty grams of the pulverized peel were transferred into 500 ml flat-bottom and macerated with 200 ml of absolute methanol for twenty-four hours and the mixture was filtered. The filtrate was concentrated and stored at -18°C in an air-tight container (Oso *et al*., 2019).

2.2.2. Identification and characterization of compounds

A concentrated extract of *A. comosus* was dissolved in methanol and the solution was used for the GC/MS analysis. The analysis was performed using Agilent Technologies GC/MS (Model 7890A) equipped with Agilent 19091IS-433HP-5MS 5% Phenyl Methyl Silox column (30 m × 250 µm × film thickness 0.25 µm) coupled with mass spectrometry. Pure helium gas as carrier gas at 1.5 mL/min constant rate was used. The injector temperature was 250 ºC. GC/MS analysis resulting in chromatogram was compared to the complete library using a data base of the National Institute of Standard and Technology (NIST). The values were presented as the relative percentage of the chemical components expressed as a percentage by peak area. The GC/MS profiling was performed at the Department of Chemical Engineering, University of Ilorin, Ilorin, Nigeria.

2.2.3. In silico Molecular docking

An *in silico* molecular docking study was done to validate the binding potency of all the compounds of *A. comosus* extract to thrombin by using AutoDock 4.2 program (Trott and Olson, 2010) and visualized with DS BIOVIA using the method described by Rizvi *et al*. (2013). The molecular dockings were conducted by using the 3D crystal structure of the PAR1, PAR3, and PAR4, obtained from the protein data bank (www.rcsb.org) (Berman *et al*., 2000) with PDB IDs 3HKJ, 2PUX, and 3QDZ respectively. The selected crystal structures were obtained from the human genome except for 2PUX which was an available murine PAR3 chosen as a human homologous (Bah *et al*., 2007). The associated thrombin and ligand complexes were deleted using DS BIOVIA. Moreover, polar hydrogen atoms were added, and the crystal water remained. The selected ligands are thrombin (PubChem CID: 65045), benzyl alcohol (PubChem CID: 244), 2-(4-methylphenyl)-indolizine (PubChem CID: 346948), and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose (PubChem CID: 542798). Benzyl alcohol, 2-(4-methylphenyl)-indolizine, and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose were selected from the GC/MS chromatogram based on their respective binding affinity with a threshold determined by thrombin, a PAR agonist. The ligands were obtained from the PubChem database (Bolton *et al*., 2008; Kim *et al*., 2019). The cubic grid box was set to $-12 \times -22 \times 20$ points with a spacing of 1.0 Å. The catalytic site of the grid box was centered on the following coordinates ($x = 68$; $y = 62$; $z = 83$) to obtain the best orientations and conformations of the ligands in the binding pockets of protein. The interaction figures were generated in both 3D and 2D to visualize the specific interactions between the selected compounds and the receptors. The docking results were recorded with binding energy and bonded residues.

2.2.4. Prediction of ADMET by computational analysis

Pharmacokinetic (PK) properties of 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-D-ribose were investigated using the pkCSM ADMET descriptors algorithm protocol (http://biosig.unimelb.edu.au/pkcsm/prediction) (Douglas *et al*., 2015). Two important chemical descriptors relate well with PK features; the absorption of drugs relies on factors such as membrane permeability [indicated by colon cancer cell line (Caco-2)], intestinal absorption, skin permeability levels, P-glycoprotein substrate or inhibitor. The distribution of drugs relies on factors such as the blood-brain barrier (logBB), CNS permeability, and the volume of distribution (VDss). Metabolism is predicted based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, and CYP2C9). Excretion is predicted based on the total clearance model and renal OCT2 substrate. The safety of compounds is

foreseen based on skin sensitization, AMES toxicity, hepatotoxicity, and hERG inhibition.

3. Results

3.1. Characterization of Phytochemical Compositions

The chromatograms of the metabolomics profiling of the volatile and semi-volatile components of the extract are presented in Figures 1 and the identified compounds are presented in Table 1.

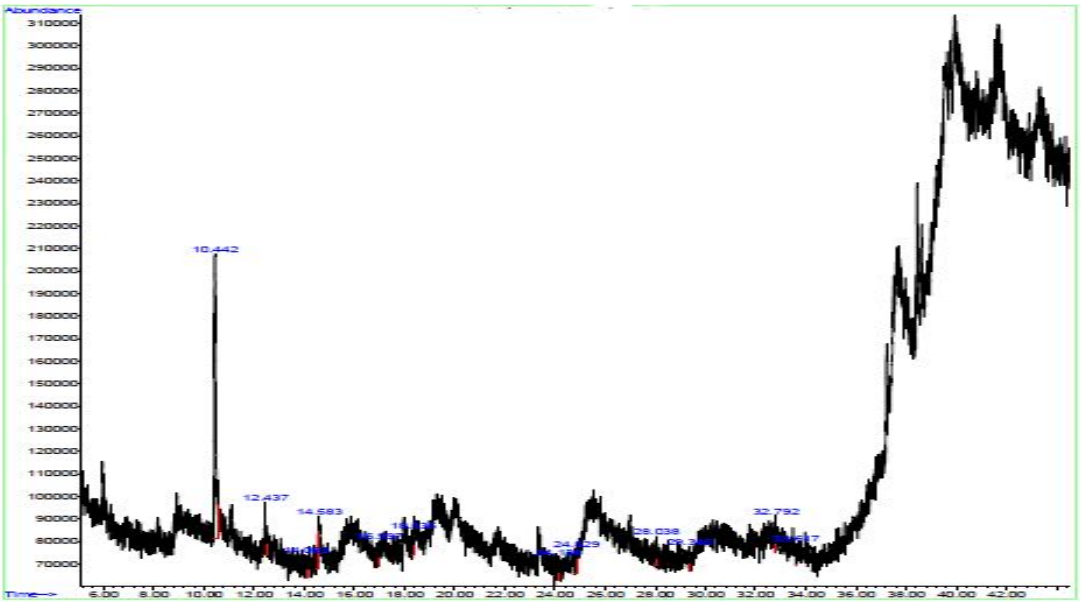

Figure 1. GC/MS chromatogram of methanol extract of the unripe peel of *A. comosus* L. with benzyl alcohol (62.52 %), 4H-1,2,4-triazol-3-amine, 4-propyl (7.12 %) and 2,5-Difluorophenylhydrazine (7.64 %) as abundant compounds

Table 1. Chemical composition of methanol extract, retention time, percentage of correlation and percentage relative composition of unripe of *A. comosus*

Compounds	RT (mins)	CM (%)	RC (%)
Benzyl alcohol	10.442	100.00	62.53
4H-1,2,4-Triazol-3-amine, 4-propyl	12.437	11.40	7.13
chloro- Acetaldehyde	14.063	3.20	2.00
2,5-Difluorophenylhydrazine	14.583	12.23	7.65
N-(3-Methylbutyl)acetamide	16.997	6.56	4.10
1,4-dinitro- Benzene	18.336	3.30	2.40
2,2-Dimethoxy-1-oxa-2-sila-1,2-dihydronaphthalene	24.134	3.66	2.29
2-p-Nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose	24.829	4.29	2.68
2,3-dihydro-2,8-dimethyl- Benz[b]-1,4-oxazepine-4(5H)-thione	28.038	3.25	2.03
3-amino-3-cyano-, methyl ester Acrylic acid	29.345	5.62	3.52
5-(ethyl) (4-diethylamino-1-methyl Pyrimidine-2,4,6 (1H,3H,5H)-trione	32.792	3.29	2.05
2-(4-methylphenyl)- Indolizine	33.617	3.12	1.95

RT=Retention time; CM= Maximum Correlation; RC= Relative composition expressed in percentage of total.

3.2. Molecular docking

The inherent nature of molecular docking is the recognition process of molecules, relating to their space and energy matching. The docking results tabulated between the PARs and the ligands are shown in Figure 2a, b, and Table 2. The results showed that 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose were found to possess the maximum binding energies as -7.0 and -6.5, -8.4 and -5.1, and -8.4 and -7.2 kcal/mol respectively towards PAR1, PAR3, and PAR4 compared to thrombin except for PAR3 where 2-(4-methylphenyl)-indolizine had lower binding energy than thrombin. However, benzyl alcohol had lower binding energy towards all the PARs compared to thrombin and had its interaction towards PAR1 despite its high percentage composition in the *A. comosus* unripe peel.

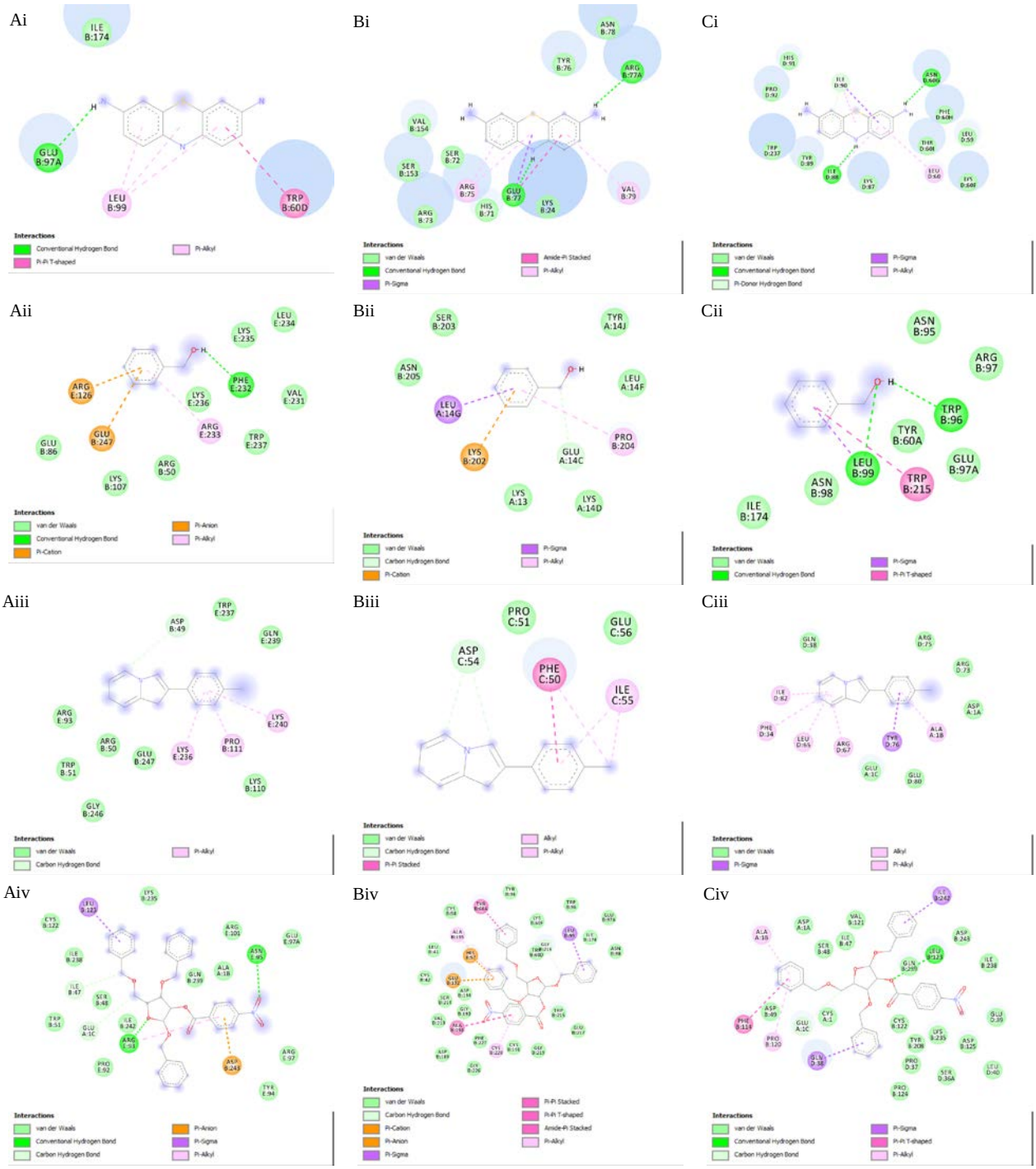

Figure 2a: 2D View of the molecular docking of PAR-1 towards and ligands: Ai thrombin, Aii benzyl alcohol, Aiii 2-(4-methylphenyl)-indolizine and Aiv 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose; PAR-3 towards and ligands: Bi thrombin, Bii benzyl alcohol, Biii 2-(4-methylphenyl)-indolizine and Biv 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose; and PAR-4 towards and ligands: Ci thrombin, Cii benzyl alcohol, Ciii 2-(4-methylphenyl)-indolizine and Civ 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose

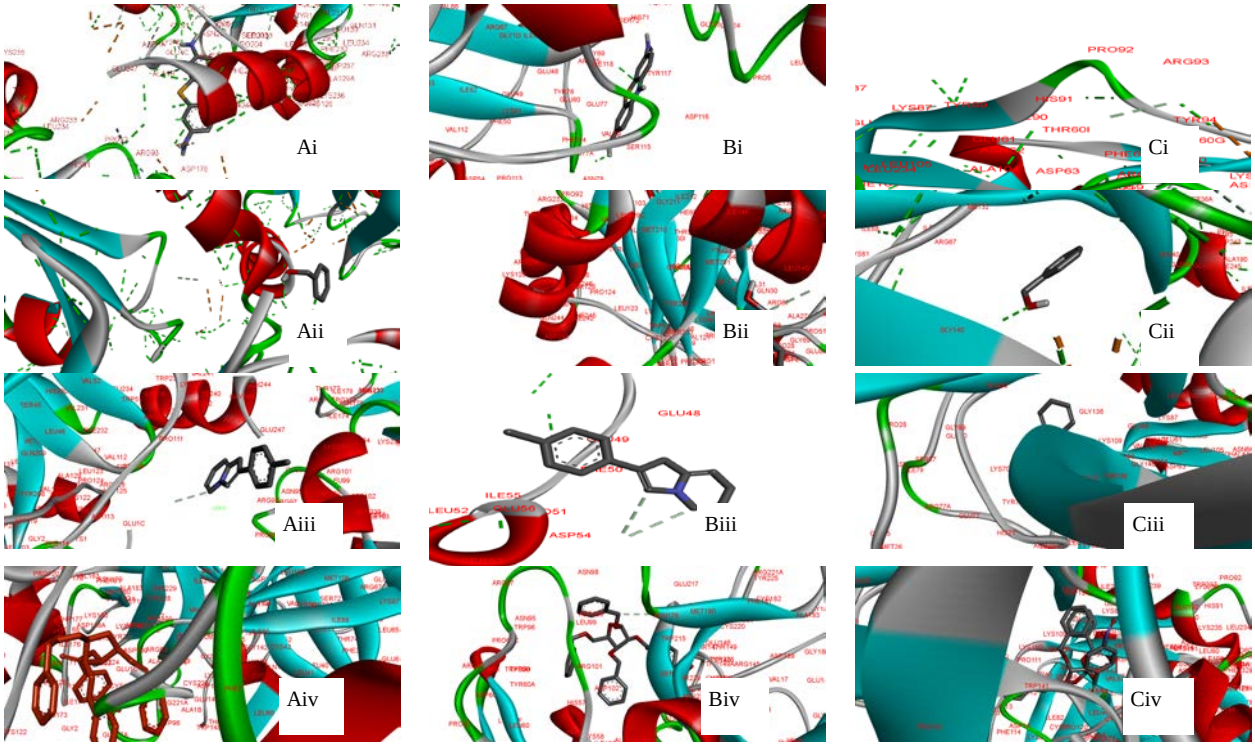

Figure 2b: 3D View of the molecular docking of PAR-1 towards and ligands: Ai thrombin, Aii benzyl alcohol, Aiii 2-(4-methylphenyl)-indolizine and Aiv 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose; PAR-3 towards and ligands: Bi thrombin, Bii benzyl alcohol, Biii 2-(4-methylphenyl)-indolizine and Biv 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose; and PAR-4 towards and ligands: Ci thrombin, Cii benzyl alcohol, Ciii 2-(4-methylphenyl)-indolizine and Civ 2-p-nitrobenzoyl-1,3,5-tribenzyl-alpha.-d-ribose

Table 2. Docking analysis of 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose from the methanolic extract of unripe of *A. comosus* L with thrombin receptors (PARs)

Ligands	PubChem ID	Molecular mass(g/mol)	Binding energy (Kcal/mol)			Amino acid residues in conventional H-bond at the binding site		
			PAR1	PAR3	PAR4	PAR1	PAR3	PAR4
Thrombin	65045	263.75	-6.1	-6.4	-6.4	Glu97	Arg 77	Asn60G, Ile88
Benzyl alcohol	244	108.14	-4.3	-5.1	-4.7	Phe232	NR	Trp96, Leu99
2-(4-methylphenyl)-indolizine	346948	209.72	-6.5	-5.1	-7.2	NR	NR	NR
2-p-nitrobenzoyl-1,3, 5-tribenzyl-α-d-ribose	542798	569.61	-7.0	-8.4	-8.4	Arg 93, Asn95	NR	Leu123

NR=No residue

3.3. Prediction of ADMET by computational analysis

The ADMET properties of 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose are presented in Table 3. 2-(4-methylphenyl)-indolizine had a Log P value of 3.91472 and was predicted to have Log P value of 3.92, water solubility (-3.99 log mol/L), Caco-2 permeability (1.65 log Papp in 10^{-6} cm/s) and 97.5 % could be absorbed through the intestine. However, 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose showed Log P of 5.86, water solubility (-5.98 log mol/L), Caco-2 permeability (1.07 log Papp in 10^{-6} cm/s) and 100 % intestinal absorption. 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose was suggested as an inhibitor of P-glycoprotein and human ether-a-go-go-related gene (hERG) and a hepatotoxic agent.

Table 3. Prediction of ADMET properties of 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose

Property	2-(4-methylphenyl)-indolizine	2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose
Absorption		
Log P	3.92	5.86
Water solubility (log mol/L)	-3.99	-5.98
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.65	1.07
Intestinal absorption (% Absorbed)	97.5	100
Skin Permeability (log Kp)	-2.11	-2.74
P-glycoprotein substrate	No	No
P-glycoprotein I inhibitor	No	Yes
P-glycoprotein II inhibitor	No	Yes
Distribution		
VDss (log L/kg)	0.20	-0.57
Fraction unbound	0.24	0.04
BBB permeability (log BB)	0.55	-1.27
CNS permeability (log PS)	-1.55	-2.93
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	Yes
CYP1A2 inhibitor	Yes	No
CYP2C19 inhibitor	Yes	Yes
CYP2C9 inhibitor	No	Yes
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	Yes
Excretion		
Total Clearance (log ml/min/kg)	0.50	0.82
Renal OCT2 substrate	No	No
Toxicity		
AMES toxicity	No	No
MTD (log mg/kg/day)	-0.72	0.84
hERG I inhibitor	No	No
hERG II inhibitor	No	Yes
Oral Rat Acute Toxicity (LD50) (mol/kg)	2.25	2.48
Oral Rat Chronic Toxicity (log mg/kg_bw/day)	0.97	1.52
Hepatotoxicity	No	Yes
Skin Sensitisation	No	No
Pyriformis toxicity (log ug/L)	0.573	0.29
Minnow toxicity (log mM)	1.7	-8.68

BBB= Blood brain barrier, CNS= Central nervous system, Cytochrome P450, Renal OCT2= organ cation transporter, MTD= Maximum tolerated dose and hERG= human Ether-à-go-go-Related Gene

4. Discussions

The metabolomic investigation on the compounds composition present in the unripe *A. comosus* methanolic extract was conducted for better clarification of the contributions of the peel to the observed reported biological activities. Several volatile compounds seen in the extract include esters, alcohols, ketones, aldehydes, and terpenes. The results showed that 12 volatile compounds are present in the unripe *A. comosus* methanolic extract. However, only 2-(4-methylphenyl)-indolizine, and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose depicted higher binding scores than thrombin. Benzyl alcohol with a relative composition of 62.52% was the prominent volatile compounds present in the extract followed by 4H-1,2,4-triazol-3-amine, 4-propyl. Benzyl alcohol, a relatively non-toxic and naturally occurring flavouring agent is usually an active compound in cosmetics (McCloskey *et al*., 1986).

The binding affinities of the identified compounds: benzyl alcohol, 2-(4-methylphenyl)-indolizine, and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose were estimated through molecular docking. The docking analysis result revealed that benzyl alcohol, the most prominent volatile compound in the extract had the least binding affinity towards PARs. This implies that the compound might not be responsible for the established antithrombotic effect of *A comosus* peel through modulation of the PARs (Limjuco *et al*., 2014; Go and Mariposque, 2018). Conversely, 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose had the best binding score towards the PARs including thrombin value with conventional hydrogen bond formation to basic residues (Asn and Arg) and van der Waal interaction in PAR1 binding site. Elokely and Doerksen (2013) reported that scoring systems generally rely on electrostatic interactions, Van der Waal's forces, and hydrophobic linkage. The conventional hydrogen interaction could be linked to the hirudin-like domain on PAR1, an exosite 1 that recruits thrombin to PAR1 (Vu *et al*., 1991). Similarly, 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose had the best effective interaction with PAR3 using the same mechanism as PAR1, but no conventional hydrogen linkage was observed. The absence of a conventional hydrogen linkage may be attributed to the differences in organism genome on the receptor. In contrast to PAR1 and PAR3, 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose could be antagonist by blocking the interaction between the receptor and G protein. This could be due to its hydrophobic nature (log P value greater than 5) and prevent the internalization of signaling in the cellular part (French and Hamilton, 2016). More so, 2-(4-methylphenyl)-indolizine had a better binding score to all the PAR1 and PAR4 with no conventional hydrogen formation. This higher binding score may be attributed to the high number of alkyl and pi alkyl bond formation suggesting other mechanisms aside hirudin-like linkage (Heuberger and Schuepbach, 2019). Also, a low binding score was observed for PAR3 for 2-(4-methylphenyl)-indolizine which may be due to organism genome difference.

The distribution, metabolic, and excretion properties of 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1, 3, 5-tribenzyl-α-d-ribose were assessed through the ADMET parameters based on the pkCSM thresholds of drug ability. The computed partition coefficient (log P) which defines the respective lipophilicity of the compounds showed that 2-(4-methylphenyl)-indolizine had relative good lipophilicity as the log P is not greater than 5. This shows that it could have good absorption due to its maintain

fitting balance maintenance between the hydrophilicity and lipophilicity suggesting good system maintenance of appropriate ligand concentration. However, 2-p-nitrobenzoyl-1, 3, 5-tribenzyl-α-d-ribose could have poor oral absorption and increased risk of promiscuity and toxicity as the log P is greater than 5 (Pajouhesh and Lenz, 2005; Hughes *et al*., 2008). Moreover, the observed lipophilicity correlates negatively to water solubility and positively to intestinal absorption. The moderate level of the lipophilicity of 2-(4-methylphenyl)-indolizine could suggest it would have no negative effect on brain exposure as indicated by the probable effect brain-blood barrier and central nervous system permeation. 2-(4-methylphenyl)-indolizine showed a comparatively better drugability score than 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose. This could also be substantiated by the inhibitory potential of 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose on P-glycoprotein and hERG. The P-glycoprotein inhibition could impair the active transport of xenobiotics in the system. Additionally, impairment in the function hERG potassium channel through inhibition may result in delayed ventricular repolarisation which could lead to a severe disturbance in the normal cardiac rhythm (Wang *et al*., 2012). The mutagenic properties computed through AMES toxicity showed the compounds are not mutagens. However, 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose was suggested to be a hepatotoxic compound. The hepatotoxic effect of 2-p-nitrobenzoyl-1, 3, 5-tribenzyl-α-d-ribose could be related to its lipophilicity and enhanced retention within the membranes and binding to non-desired protein.

5. Conclusion

This study identified 2-(4-methylphenyl)-indolizine and 2-p-nitrobenzoyl-1,3,5-tribenzyl-α-d-ribose as potential inhibitors of PARs. They could perchance function additively in modulating the signaling event, leading to clot formation. Their therapeutic use as anti-thrombotic factors may lead to a beneficial solution against coronary atherothrombotic diseases. Further investigations on the potential toxicity of the phytocompounds through various laboratory studies are recommended.

Conflict of Interest

The authors declare that they have no conflict of interestS.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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References

Abdel-Mawgoud BM, Khedr FG and Mohammed EI. 2019. Phenolic Compounds, Antioxidant and Antibacterial Activities of *Rhus flexicaulis*. *Joradn J Biol Sci.* **12**(1):17-21

Adams MN, Ramachandran R, Yau MK, Suen JY, Fairlie DP, Hollenberg MD and Hooper JD. 2011. Structure, function and pathophysiology of protease activated receptors. *Pharmacol Ther.* **130**: 248-282.

Ashorobi D and Fernandez R. 2019. **Thrombosis**. Treasure Island StartPearls publishing.

Azevedo AP, Farias JC, Costa GC, Ferreira SC, Aragão-Filho WC, Sousa PR, Pinheiro MT, Maciel MC, Silva LA, Lopes AS, Barroqueiro ES, Borges MO, Guerra RN and Nascimento FR. 2007. Anti-thrombotic effect of chronic oral treatment with *Orbignya phalerata* Mart. *J Ethnopharmacology*. **111**(1): 155-159.

Bah A, Chen Z, Bush-Pelc LA, Mathews FS and Di Cera E. 2007. Crystal structures of murine thrombin in complex with the extracellular fragments of murine protease-activated receptors PAR3 and PAR4. *PNAS.* **104**(28): 11603-11608.

Banji A, Goodluck B, Oluchi O and Stephen F. 2018. Antimicrobial and Antioxidant Activities of Crude Methanol Extract and Fractions of *Andrographis paniculata* leaf (Family: Acanthaceae) (Burm. f.) Wall. Ex Nees. *Jordan J Biol Sci.* **11**(1): 23-30

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE. 2000. The Protein Data Bank. *Nucleic Acids Res.* **28**(1): 235-242.

Bolton EE, Tiessen PA and Bryant SH. 2008. PubChem: integrated platform of small molecules and biological activities. *Annu Rep Comput Chem.* **4**: 217-241.

Charlotte M, Julia N, Danby S, Katie RZ, Leo N, Kami P, Badr K, Sven S, Niklas B, Danny K, Thomas JS, Ludwig TW, Joachim P, Markus M, Michael J, Walter D, Tom A, Franz-Josef N, Anthony HG, Jurrien MB, Michael L and Konstantin S. 2019. Eosinophil-platelet interactions promote atherosclerosis and stabilize thrombosis with eosinophil extracellular traps. *Blood.* **134**(21): 1859-1872.

Coughlin SR. 2000. Thrombin signalling and protease-activated receptors. *Nature*. **407**: 258-64.

Covic L, Gresser AL, Talavera J, Swift S and Kuliopulos A 2002. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl Acad Sci*. **99**: 643-648.

da Silva L and Ferreira RM. 2015. Novel Anticoagulants in Non-Valvular Atrial Fibrillation: An Evidence- Based Analysis. *Evidence Based Medicine and Practice*. **1(1):** 101-102.

De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, Baigent C, Huber K, Jespersen J, Kristensen SD, Lip GY, Morais J, Rasmussen LH, Siegbahn A, Verheugt FW and Weitz JI. 2013. Vitamin K antagonists in heart disease: current status and perspectives (Section III). Position paper of the ESC Working Group on Thrombosis-Task Force on Anticoagulants in Heart Disease. *Thromb Haemost*. **110(6):** 1087-107.

Douglas EVP, Tom LB and David BA. 2015. pkCSM: predicting small-molecule pharmacokinetic properties using graph-based signatures. *J Medicinal Chemistry*. **58(9):** 4066–4072.

Elokely KM and Doerkse RJ. 2013. Docking challenge: protein sampling and molecular docking performance. *J Chemical Information and Modeling*. **53**(8): 1934-1945.

Evangelista JH, De Vera MJ, Garcia RS. Joven MG, Nerosa MJA and Solidum JN. 2012. Preliminary Assessment of In vitro Anticoagulant Activity vs. Heparin 1,000I.U. and Cytotoxicity of Selected Philippine Medicinal Plants. *Int J Chemical and Environmental Engineering*. **3**(6): 371-376.

French SL and Hamilton JR. 2016. Protease-activated receptor 4: From structure to function and back again. *Br. J. Pharmacol*, **173**: 2952-2965.

Go CEO and Mariposque JRA. 2018. **Hematological effects of pineapple *Ananas comosus L. Merr* peel extracts and commercial Bromelain on male albino mice (*Mus musculus*)**. Herdin Record#: R07-USC-18082909314518

Gryka RJ, Buckley LF and Anderson SM. 2017. Vorapaxar: The current role and future directions of a novel protease-activated receptor antagonist for risk reduction in atherosclerotic disease. *Drugs R D.* **17**: 65-72

Herrington W, Lacey B, Sherliker P, Armitage J and Lewington S. 2016. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circulation Res.* **118**(4): 535-546.

Heuberger DM and Schuepbach RA. 2019. Protease-activated receptors (PARs): mechanisms of action and potential therapeutic modulators in PAR-driven inflammatory diseases. *Thrombosis J*. **17**(4): 1-24.

Hollenberg MD and Compton SJ. 2002. International union of pharmacology.Proteinase-activated receptors. *Pharmacol Rev*. **54**: 203-217.

Hughes JD, Blagg J, Price DA, Bailey S, Decrescenzo GA, Devraj RV, Ellsworth E, Fobian YM, Gibbs ME, Gilles RW, Greene N, Huang E, Krieger-Burke T, Loesel J, Wager T, Whiteley L and Zhang Y. 2008. Physiochemical drug properties associated with in vivo toxicological outcomes. *Bioorg Med Chem Lett*. **18**: 48725.

Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J and Bolton EE. 2019. PubChem 2019 update: improved access to chemical data. *Nucleic acids Res.* **47**(1): 1102–1109.

Ko HH, Hsieh HK, Liu CT, Lin CH, Teng CM and Lin CN. 2004. Structure-activity relationship studies on chalcone derivatives: The potent inhibition of platelet aggregation. *J Pharm Pharmacol*. **56**: 1333-7.

Lau AJ, Toh DF, Chua TK, Pang YK, Woo SO and Koh HL. 2009. Antiplatelet and anticoagulant effects of *Panax notoginseng*: comparison of raw and steamed *Panax notoginseng* with *Panax ginseng* and *Panax quinquefolium. J Ethnopharmacology*. **125**(3): 380-386.

Lee W, Ku SK and Bae JS. 2015. Antiplatelet, anticoagulant and profibrinolytic activities of baicalin. *Arch Pharm Res.* **38:** 893-903.

Li T, Shen P, Liu W, Liu C, Liang R, Yan N, Chen J. 2014. Major polyphenolics in pineapple peels and their antioxidant interactions. *Int J Food Prop*. **17**, 1805–1817.

Limjuco RP, Catalan MP and Aquino FC. 2014. Anticoagulant Activity of Pineapple (Ananas comosus) Extract on Human Blood Samples. *IAMURE Int J Sci Clin Lab.* **6**(1).

Ma L, Perini R, McKnight W, Dicay M, Klein A, Hollenberg MD, Wallace JL (2005). Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets. *Proc Natl Acad Sci*. **102**: 216-220.

Mahmud S, Samina A, Rahman MA, Aklima J, Akhter S, Merry SR, Jubair SMR, Dash R and Emran TB. 2015. Antithrombotic Effects of Five Organic Extracts of Bangladeshi Plants In Vitro and Mechanisms in In Silico Models. *Evidence-Based Compl Alt Med.* **782742**: 1-8.

McCloskey SE, Gershanik JJ, Lertora JJ, White L and George WJ. 1986. Toxicity of benzyl alcohol in adult and neonatal mice. *J Pharm Sci*. **75**(7): 702-705.

Mohd Nor NH, Othman F, Mohd Tohit ER and Md Noor S. 2016. Medicinal herbals with antiplatelet properties benefit in coronary atherothrombotic diseases. *Thrombosis*. 5952910.

Mumaw MM, De La Fuente M, Arachiche A, Wahl JK and Nieman MT. 2015. Development and characterization of monoclonal antibodies against Protease Activated Receptor 4 (PAR4). *Thromb Res*. **135**: 1165-1171.

Oso BJ, Oyewo EB and Oladiji AT. 2019. Influence of ethanolic extracts of dried fruit of Xylopia aethiopica (Dunal) A. Rich on haematological and biochemical parameters in healthy Wistar rats. *Clin Phyt.* **5**: 9

Pajouhesh H and Lenz GR. 2005. Medicinal chemical properties of successful central nervous system drugs. *Neuro RX*. **2**: 54153

Rizvi SMD, Shakil S and Haneef M. 2013. A simple click by click protocol to perform docking: autodock 4.2 made easy for non-bioinformaticians. *EXCLI J*. **12**: 831-857.

Shikha J, Dangi CBS, Kaur M, Singh H, Peter J and Kosta S. 2014. Plants as anticoagulant and antithrombotic agents. *World J Pharm Res*. **3**(1): 4573-4585.

Trott O and Olson AJ. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem*. **31**(2): 455-461.

Vu TK, Wheaton VI, Hung DT, Charo I and Coughlin SR. 1991. Domains specifying thrombin-receptor interaction. *Nature*. **353**: 674-677

Wang S, Li Y, Wang J, Chen L, Zhang L, Yu H and Hou T. 2012. ADMET evaluation in drug discovery. 12. Development of binary classification models for prediction of hERG potassium channel blockage. *Mol Pharm*. **9**(4): 996-1010.

WHO (2017). "Cardiovascular Diseases report". Accessed April 4, 2020.

Wong PC, Seiffert D, Bird JE, Watson CA, Bostwick JS, Giancarli M, Allegretto N, Hua J, Harden D and Guay J. 2017. Blockade of protease-activated receptor-4 (PAR4) provides robust antithrombotic activity with low bleeding. *Sci Transl Med*. **9**: eaaf5294.

Yoo H, Ku SK, Lee W, Kwak S, Baek YD, Min BW, Jeong G and Bae J. 2014. Antiplatelet, anticoagulant, and profibrinolytic activities of *cudratricus xanthone* A. *Arch Pharm Res*. **37**: 1069-1078.